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ScienceDirect
Acta Biomaterialia 5 (2009) 2331–2337
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Preparation and in vitro evaluation of plasma-sprayed Mg_2SiO_4 coating on titanium alloy

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Received 24 November 2008; received in revised form 5 March 2009; accepted 5 March 2009
Available online 13 March 2009

Abstract

In this paper, chemically synthesized Mg_2SiO_4 (MS) powder was plasma-sprayed onto a titanium alloy substrate to evaluate its application potentials in biomedicine. The phase composition and surface morphology of the MS coating were analyzed. Results showed that the MS coating was composed mainly of Mg_2SiO_4 phase, with a small amount of MgO and glass phases. Mechanical testing showed that the coating exhibited good adhesion strength to the substrate due to the close thermal expansion coefficient between the MS ceramic and the titanium alloy substrate. The measured bonding strength was as high as 41.5 ± 5.3 MPa, which is much higher than the traditional HA coating. In vitro cytocompatibility evaluation of the MS coating was performed using canine bone marrow stem cells (MSCs). The MSCs exhibited good adhesion, proliferation and differentiation behavior on the MS coating surface, which can be explained by the high protein adsorption capability of the MS coating, as well as the stimulatory effects of Mg and Si ions released from the coating. The proliferation rate of the MSCs on MS coating was very close to that on the hydroxylapatite (HA) coating. Alkaline phosphatase (ALP) activity analysis demonstrated that the ALP level of the MSCs on the MS coating remained high even after 21 days, implying that the surface characteristics of the coating are beneficial for the differentiation of MSCs. In summary, our results suggest that MS coating might be a new approach to prepare bone implants.

Keywords: Mg_2SiO_4; Coating; Bone marrow stem cell; Hard tissue replacement

1. Introduction

Silicon is an essential trace element in animal nutrition and has a very important function in the early stage of bone and ligament tissue formation [1]. Modern molecular biological techniques demonstrate that more than 60 genes are silicon sensitive [2]. Hydrated silica gel can enhance the proliferation of osteoblasts and activate the production of transforming growth factors [3,4]. A lot of silicate-containing glasses or ceramics have proved to be biocompatible and able to chemically bond with bone tissue [5–8]. Plasma-sprayed $CaO–SiO_2$-based ceramic coatings show not only good biocompatibility but also excellent bonding strength with titanium alloy substrate, even though their degradation in a biological environment affects the fixation of the implant to the host bone tissue [8].

Magnesium is naturally present in bony tissues and is essential to human metabolism [9–11]. It is a co-factor for many enzymes, and also stabilizes the structures of DNA and RNA [11]. Because of its functional roles, magnesium is classified as an essential minor element [12]. Many researchers have demonstrated the stimulatory effects of magnesium on the growth of new bony tissues [13–15]. The interfacial strength of a hydroxylapatite (HA)-coated implant with bone tissue can be enhanced

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doi:10.1016/j.actbio.2009.03.003

by enriched magnesium in the coating [16]. This increased bonding strength with bone tissue contributed to the enhancement of the adhesion of the osteoblasts on the implant surface modified by magnesium. Zreiqat et al. [13] found that cells grown on magnesium-enriched substrate expressed a significantly increased level of α5β1 integrin receptor and enhanced expression of collagen I extracellular matrix protein. Two studies conducted by Yamasaki et al. [14,15] reported similar beneficial effects of magnesium by observing the cellular behavior on magnesium-enriched apatite or collagen materials.

$MgO–SiO_2$-based ceramics have also been widely studied for their applications in biomedicine. It was found that the differentiation of human bone marrow stem cells (MSCs) cultured on akermanite ceramics was promoted by the up-regulation of osteogenic gene expression [17]. Wu and Chang [18] studied the effects of the degradation products of diopside, akermanite and bredigite bioceramics on the proliferation of osteoblasts. The Mg and Si ionic products of these ceramics clearly stimulated cell proliferation at low concentrations. Enstatite has been reported as a new machinable biomaterial for use in dental and orthopedic prostheses applications [19]. The good cytocompatibility of forsterite ceramic has also been demonstrated [20]. Osteoblasts were found to adhere and proliferate well on the sintered forsterite surface.

In this paper, Mg_2SiO_4 (MS) ceramic was used as a plasma-spraying feedstock. The phase composition and surface morphology of the coating were evaluated by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The bonding strength of the coating with titanium alloy substrate was measured. To investigate the cytocompatibility of the coating, canine bone MSCs were cultured on the coating surface and the adhesion, spreading, proliferation and differentiation behavior of the cells were studied in comparing with the traditional HA coating.

2. Experimental details

2.1. Preparing the MS coating

MS powder was prepared by the sol–gel process according to reported method [20]. Reagent-grade magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$) and colloidal SiO_2 was mixed at a molar ratio of 2 to 1 by vigorous stirring at room temperature for 3 h, then aged for 24 h. After gelating overnight at 60 °C, the wet gel was subsequently dried at 120 °C for 48 h. The obtained dry gel was then milled for 24 h and sieved to 300-mesh, followed by calcining at 1300 °C for 3–5 h. After cooling naturally, the as-sintered powder was abrasive and sieved to 250-mesh for plasma spraying onto Ti–6Al–4V plates (10 × 10 × 2 mm) by vacuum plasma spraying (VPS) (Sulzer Metco, Switzerland). The detailed plasma-spraying parameters are shown in Table 1. HA coating prepared by VPS served as the control.

Table 1
Plasma spraying parameters.

Argon plasma gas flow rate (slpm)	40
Hydrogen plasma gas flow rate (slpm)	10
Spray distance (mm)	280
Argon powder carrier gas (slpm)	3.5
Current (A)	650
Voltage (V)	68
Powder feed rate (g min^{-1})	18

2.2. Characteristics and bonding strength with titanium alloy substrate

The phase of the feedstock and coating was determined by X-ray diffraction (D/max 2550v, Japan) using Cu K_α X-radiation. The surface morphology was observed by SEM (EPMA-8705QH2, Japan). Surface roughness (Ra) was measured by a profilometer (Hommelwerke T8000-C, Germany).

The standard method designated as ASTM C 633 [21] was employed to measure the bonding strength of the MS coating on the titanium alloy substrate. Briefly, two identical cylindrical Ti–6Al–4V rods (ϕ25.4 × 25.4 mm) underwent two different treatments. One was coated with MS (about 400 μm) and the other was gritblast-roughened to enhance resin. A thin layer of adhesive glue (E-7, Shanghai, China) was applied to bond the two cylinders together. The tensile bonding strength was measured using a materials tester (Instron-5592, SATEC, USA) and the average of five measurements was recorded as the bonding strength.

2.3. In vitro cytocompatibility

Canine bone MSCs were obtained by a conventional explanting technique documented elsewhere [22]. Cells, suspended in 200 μl of Dulbecco's modified Eagle's medium (supplemented with 10% fetal bovine serum, 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin) at a density of 5×10^4 cells ml^{-1} (passages 5–7), were seeded onto a 1 cm^2 sample sterilized by autoclaving at 120 °C for 30 min. The culture was kept at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity. The culture medium was changed every other day. After cultivation for 2, 4 and 6 days, the samples were taken out from the culture plates and fixed with 2.5% glutaradehyde in a 0.1 M sodium cacodylate buffer (pH 7.4) for 1 h. After rinsing three times with phosphate-buffered saline, 10 min each time, and dehydrating sequentially in a series of ethanol (50%, 70%, 95% and 100%), each concentration twice for 10 min each time, the adhesion and spreading of the cells on the MS coating were observed by SEM.

The MTT assay was applied to determine the viability and proliferation of the MSCs. On complete removal of the original culture medium, 720 μl of fresh culture medium and 80 μl of MTT solution (5 mg ml^{-1}) were added to each well. The plate was incubated under the same cultivation conditions for another 4 h, then an equal volume

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